

WHAT IS CLAIMED IS:

- 1 1. An isolated infectious human-bovine chimeric parainfluenza virus
2 (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a
3 large polymerase protein (L), and a partial or complete PIV background genome or
4 antigenome of a human PIV (HPIV) or bovine PIV (BPIV) combined with one or more
5 heterologous gene(s) or genome segment(s) of a N, P, L, or M gene of a different PIV to
6 form a human-bovine chimeric PIV genome or antigenome.
- 1 2. The chimeric PIV of claim 1, wherein said one or more
2 heterologous gene(s) or genome segment(s) encodes one or more PIV N, P, C, D, V, M,
3 F, HN and/or L protein(s) or fragment(s) thereof.
- 1 3. The chimeric PIV of claim 1, wherein said one or more
2 heterologous gene(s) or genome segment(s) encodes a complete open reading frame
3 (ORF) of one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s).
- 1 4. The chimeric PIV of claim 1, wherein said one or more
2 heterologous gene(s) or genome segment(s) includes a heterologous regulatory element
3 comprising an extragenic 3' leader or 5' trailer region, a gene-start signal, gene-end
4 signal, RNA editing site, encapsidation signal, intergenic region, or 3' or 5' non-coding
5 region.
- 1 5. The chimeric PIV of claim 1, wherein said background genome or
2 antigenome incorporates a heterologous genome segment integrated with the background
3 genome or antigenome to form a chimeric gene.
- 1 6. The chimeric PIV of claim 1, wherein a heterologous gene or
2 genome segment is added adjacent to or within a noncoding region of the partial or
3 complete PIV background genome or antigenome.
- 1 7. The chimeric PIV of claim 1, wherein a heterologous gene or
2 genome segment is added or substituted at a position corresponding to a wild-type gene
3 order position of a counterpart gene or genome segment within the partial or complete
4 PIV background genome or antigenome.

1 8. The chimeric PIV of claim 1, wherein a heterologous gene or
2 genome segment is added or substituted at a position that is more promoter-proximal or
3 promoter-distal compared to a wild-type gene order position of a counterpart gene or
4 genome segment within the partial or complete PIV background genome or antigenome.

1 9. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome comprises a partial or complete HPIV background genome or antigenome
3 combined with one or more heterologous genes or genome segments from a BPIV.

1 10. The chimeric PIV of claim 9, wherein the partial or complete HPIV
2 background genome or antigenome is combined with one or more heterologous gene(s) or
3 genome segment(s) of a N, P, L, or M gene of a BPIV to form a human-bovine chimeric
4 PIV genome or antigenome.

1 11. The chimeric PIV of claim 9, wherein said one or more
2 heterologous gene(s) or genome segment(s) encodes a complete open reading frame
3 (ORF) of one or more PIV N, P, L, or M protein(s).

1 12. The chimeric PIV of claim 9, wherein a bovine PIV3 N, M, L, or P
2 open reading frame (ORF) is substituted for a human PIV3 N, M, L, or P ORF to form the
3 chimeric PIV.

1 13. The chimeric PIV of claim 9, wherein said one or more
2 heterologous gene(s) or genome segment(s) includes a heterologous regulatory element
3 comprising an extragenic 3' leader or 5' trailer region, a gene-start signal, gene-end
4 signal, editing region, intergenic region, or a 3' or 5' non-coding region.

1 14. The chimeric PIV of claim 9, wherein said heterologous gene or
2 genome segment encodes a bovine PIV3 M, L, or P protein.

1 15. The chimeric PIV of claim 9, which is selected from rHPIV3-M_B,
2 rHPIV3-L_B, or rHPIV3-P_B.

1 16. The chimeric PIV of claim 9, wherein the chimeric genome or
2 antigenome is further modified by addition or substitution of one or more additional
3 heterologous gene(s) or genome segment(s) from a bovine PIV within the partial or

4 complete bovine background genome or antigenome to increase genetic stability or alter
5 attenuation, reactogenicity or growth in culture of the chimeric virus.

1 17. The chimeric PIV of claim 1, wherein the genome or antigenome is
2 further modified by introduction of one or more attenuating mutations identified in a
3 biologically derived mutant PIV or other mutant nonsegmented negative stranded RNA
4 virus.

1 18. The chimeric PIV of claim 17, wherein the genome or antigenome
2 incorporates at least one and up to a full complement of attenuating mutations present
3 within PIV3 JS *cp45*.

1 19. The chimeric PIV of claim 17, wherein the genome or antigenome
2 incorporates at least one and up to a full complement of attenuating mutations specifying
3 an amino acid substitution in the L protein at a position corresponding to Tyr₉₄₂, Leu₉₉₂,
4 or Thr₁₅₅₈ of JS; in the N protein at a position corresponding to residues Val₉₆ or Ser₃₈₉ of
5 JS, in the C protein at a position corresponding to Ile₉₆ of JS, in the M protein at a
6 position corresponding to residues Pro₁₉₉ of JS, in the F protein at a position
7 corresponding to residues Ile₄₂₀ or Ala₄₅₀ of JS, in the HN protein at a position
8 corresponding to residue Val₃₈₄ of JS, a nucleotide substitution a 3' leader sequence of the
9 chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS, and/or a
10 mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS.

1 20. The chimeric PIV of claim 17, wherein the genome or antigenome
2 incorporates attenuating mutations from different biologically derived mutant PIVs.

1 21. The chimeric PIV of claim 17, wherein the genome or antigenome
2 incorporates an attenuating mutation at an amino acid position corresponding to an amino
3 acid position of an attenuating mutation identified in a heterologous, mutant negative
4 stranded RNA virus.

1 22. The chimeric PIV of claim 17, wherein the genome or antigenome
2 includes at least one attenuating mutation stabilized by multiple nucleotide changes in a
3 codon specifying the mutation.

1 23. The chimeric PIV of claim 1, wherein the genome or antigenome
2 comprises an additional nucleotide modification specifying a phenotypic change selected
3 from a change in growth characteristics, attenuation, temperature-sensitivity, cold-
4 adaptation, plaque size, host-range restriction, or a change in immunogenicity.

1 24. The chimeric PIV of claim 23, wherein the additional nucleotide
2 modification alters one or more of the PIV N, P, C, D, V, M, F, HN and/or L genes
3 and/or a 3' leader, 5' trailer RNA editing site, encapsidation signal, and/or an intergenic
4 region.

1 25. The chimeric PIV of claim 23, wherein one or more genes of the
2 chimeric virus is deleted in whole or in part or expression of the genes is reduced or
3 ablated by a mutation in an RNA editing site, by a frameshift mutation, by a mutation that
4 alters an amino acid specified by an initiation codon, or by introduction of one or more
5 stop codons in an open reading frame (ORF) of the gene.

1 26. The chimeric PIV of claim 23, wherein a modification is
2 introduced in the chimeric genome or antigenome comprising a partial or complete
3 deletion of one or more C, D and/or V ORF(s) or one or more nucleotide change(s) that
4 reduces or ablates expression of said one or more C, D and/or V ORF(s).

1 27. The chimeric PIV of claim 17, wherein the chimeric genome or
2 antigenome is modified to encode a non-PIV molecule selected from a cytokine, a T-cell
3 helper epitope, a restriction site marker, or a protein of a microbial pathogen capable of
4 eliciting a protective immune response in a mammalian host.

1 28. The chimeric PIV of claim 1, wherein the bovine-human chimeric
2 genome or antigenome comprises a partial or complete PIV vector genome or antigenome
3 combined with one or more heterologous genes or genome segments encoding one or
4 more antigenic determinants of one or more heterologous pathogens.

1 29. The chimeric PIV of claim 28, wherein said one or more
2 heterologous pathogens is a heterologous PIV and said heterologous gene(s) or genome
3 segment(s) encode(s) one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s) or
4 fragment(s) thereof.

1 30. The chimeric PIV of claim 28, wherein the vector genome or
2 antigenome is a partial or complete HPIV genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more
4 heterologous PIV(s).

1 31. The chimeric PIV of claim 30, wherein said one or more
2 heterologous PIV(s) is/are selected from HPIV1, HPIV2, or HPIV3.

1 32. The chimeric PIV of claim 30, wherein the vector genome or
2 antigenome is a partial or complete HPIV genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more
4 heterologous HPIV(s).

1 33. The chimeric PIV of claim 32, wherein the vector genome or
2 antigenome is a partial or complete HPIV3 genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more
4 heterologous HPIV(s).

1 34. The chimeric PIV of claim 33, wherein the chimeric genome or
2 antigenome incorporates one or more gene(s) or genome segment(s) of a BPIV that
3 specifies attenuation.

1 35. The chimeric PIV of claim 31, wherein one or more HPIV1 or
2 HPIV2 gene(s) or genome segment(s) encoding one or more HN and/or F glycoprotein(s)
3 or antigenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated
4 within the partial or complete HPIV3 vector genome or antigenome.

1 36. The chimeric PIV of claim 35, wherein both HPIV1 genes
2 encoding HN and F glycoproteins are substituted for counterpart HPIV3 HN and F genes
3 to form a chimeric HPIV3-1 vector genome or antigenome which is further modified by
4 addition or incorporation of one or more gene(s) or gene segment(s) encoding one or
5 more antigenic determinant(s) of HPIV2 and one or more heterologous gene(s) or genome
6 segment(s) of a BPIV that specifies attenuation.

1 37. The chimeric PIV of claim 35, wherein a transcription unit
2 comprising an open reading frame (ORF) of an HPIV2 HN gene is added to or
3 incorporated within the chimeric HPIV3-1 vector genome or antigenome.

1 38. The chimeric PIV of claim 32, wherein a plurality of antigenic
2 determinants of multiple HPIVs are added to or incorporated within the partial or
3 complete HPIV vector genome or antigenome.

1 39. The chimeric PIV of claim 35, wherein the vector genome or
2 antigenome is a partial or complete HPIV genome or antigenome and the heterologous
3 pathogen is selected from measles virus, subgroup A and subgroup B respiratory
4 syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human
5 immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein
6 Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

1 40. The chimeric PIV of claim 39, wherein said one or more
2 heterologous antigenic determinant(s) is/are selected from measles virus HA and F
3 proteins, subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins,
4 mumps virus HN and F proteins, human papilloma virus L1 protein, type 1 or type 2
5 human immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus
6 gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein
7 Barr Virus gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus E and
8 NS1 proteins, and alphavirus E protein, and antigenic domains, fragments and epitopes
9 thereof.

1 41. The chimeric PIV of claim 40, wherein the heterologous pathogen
2 is measles virus and the heterologous antigenic determinant(s) is/are selected from the
3 measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1 42. The chimeric PIV of claim 41, wherein a transcription unit
2 comprising an open reading frame (ORF) of a measles virus HA gene is added to or
3 incorporated within a HPIV3 vector genome or antigenome.

1 43. The chimeric PIV of claim 40, which incorporates a gene or
2 genome segment from respiratory syncytial virus (RSV).

1 44. The chimeric PIV of claim 43, wherein the gene or genome
2 segment encodes a RSV F and/or G glycoprotein or immunogenic domain(s) or epitope(s)
3 thereof.

1 45. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome comprises a partial or complete BPIV background genome or antigenome
3 combined with one or more heterologous gene(s) or genome segment(s) from a human
4 PIV.

1 46. The chimeric PIV of claim 45, wherein one or more HPIV
2 glycoprotein genes selected from HN and F, or one or more genome segments encoding a
3 cytoplasmic domain, transmembrane domain, ectodomain or immunogenic epitope
4 thereof, is/are substituted for one or more counterpart genes or genome segments within
5 the BPIV background genome or antigenome.

1 47. The chimeric PIV of claim 1 which is a virus.

1 48. The chimeric PIV of claim 1 which is a subviral particle.

1 49. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 1 combined with a
4 physiologically acceptable carrier.

1 50. The method of claim 49, wherein the chimeric PIV is administered
2 in a dose of 10^3 to 10^7 PFU.

1 51. The method of claim 49, wherein the chimeric PIV is administered
2 to the upper respiratory tract.

1 52. The method of claim 49, wherein the chimeric PIV is administered
2 by spray, droplet or aerosol.

1 53. The method of claim 49, wherein the chimeric PIV and a second
2 attenuated PIV are administered simultaneously as a mixture.

1 54. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 9 combined with a
4 physiologically acceptable carrier.

1 55. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 17 combined with a
4 physiologically acceptable carrier.

1 56. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 23 combined with a
4 physiologically acceptable carrier.

1 57. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 28 combined with a
4 physiologically acceptable carrier.

1 58. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 1 in
3 a physiologically acceptable carrier.

1 59. The immunogenic composition of claim 58, formulated in a dose of
2 10^3 to 10^7 PFU.

1 60. The immunogenic composition of claim 58, formulated for
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1 61. The immunogenic composition of claim 58, wherein the chimeric
2 PIV elicits an immune response against one or more virus(es) selected from HPIV1,
3 HPIV2 and HPIV3.

1 62. The immunogenic composition of claim 61, wherein the chimeric
2 PIV elicits an immune response against HPIV3 and another virus selected from HPIV 1,
3 HPIV2 and HPIV3.

1 63. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 9 in
3 a physiologically acceptable carrier.

1 64. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 17 in
3 a physiologically acceptable carrier.

1 65. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 23 in
3 a physiologically acceptable carrier.

1 66. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 28 in
3 a physiologically acceptable carrier.

1 67. An isolated polynucleotide molecule comprising a partial or
2 complete PIV background genome or antigenome of a human PIV (HPIV) or bovine PIV
3 (BPIV) combined with one or more heterologous gene(s) or genome segment(s) of a N, P,
4 L, or M gene of a different PIV to form a human-bovine chimeric PIV genome or
5 antigenome.

1 68. The isolated polynucleotide molecule of claim 67, wherein said one
2 or more heterologous gene(s) or genome segment(s) encodes one or more PIV N, P, L, or
3 M protein(s) or fragment(s) thereof.

1 69. The isolated polynucleotide molecule of claim 67, wherein said one
2 or more heterologous gene(s) or genome segment(s) encodes a complete open reading
3 frame (ORF) of one or more PIV N, P, L, or M protein(s).

1 70. The isolated polynucleotide molecule of claim 67, wherein said one
2 or more heterologous gene(s) or genome segment(s) includes a heterologous regulatory

3 element comprising an extragenic 3' leader or 5' trailer region, a gene-start signal, gene-
4 end signal, editing region, encapsidation signal, intergenic region, or 3' or 5' non-coding
5 region.

1 71. The isolated polynucleotide molecule of claim 67, wherein the
2 chimeric genome or antigenome comprises a partial or complete HPIV background
3 genome or antigenome combined with one or more heterologous genes or genome
4 segments from a BPIV.

1 72. The isolated polynucleotide molecule of claim 71, wherein the
2 partial or complete HPIV background genome or antigenome is combined with one or
3 more heterologous gene(s) or genome segment(s) of a N, P, L, or M gene of a BPIV to
4 form a human-bovine chimeric PIV genome or antigenome.

1 73. The isolated polynucleotide molecule of claim 71, wherein said one
2 or more heterologous gene(s) or genome segment(s) encodes a complete open reading
3 frame (ORF) of one or more PIV N, P, L, or M protein(s).

1 74. The isolated polynucleotide molecule of claim 71, wherein a
2 bovine PIV3 N, L, M or P open reading frame (ORF) is substituted for a human PIV3 N,
3 L, M, or P ORF to form the chimeric PIV.

1 75. The isolated polynucleotide molecule of claim 71, wherein said one
2 or more heterologous gene(s) or genome segment(s) includes a heterologous regulatory
3 element comprising an extragenic 3' leader or 5' trailer region, a gene-start signal, gene-
4 end signal, editing region, intergenic region, or a 3' or 5' non-coding region.

1 76. The isolated polynucleotide molecule of claim 71, wherein said
2 heterologous gene or genome segment encodes a bovine PIV3 M, L, or P protein.

1 77. The isolated polynucleotide molecule of claim 76, wherein said
2 heterologous gene or genome segment encodes a bovine PIV3 M protein.

1 78. The isolated polynucleotide molecule of claim 76, wherein said
2 heterologous gene or genome segment encodes a bovine PIV3 P protein.

1 79. The isolated polynucleotide molecule of claim 76, wherein said
2 heterologous gene or genome segment encodes a bovine PIV3 L protein.

1 80. The isolated polynucleotide molecule of claim 67, wherein the
2 chimeric genome or antigenome is further modified by one or more attenuating
3 mutations.

1 81. The isolated polynucleotide molecule of claim 67, further
2 comprising a nucleotide modification specifying a phenotypic change selected from a
3 change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation,
4 plaque size, host-range restriction, or a change in immunogenicity.

1 82. A method for producing an infectious attenuated chimeric PIV
2 particle from one or more isolated polynucleotide molecules encoding said PIV,
3 comprising:

4 expressing in a cell or cell-free lysate an expression vector comprising an
5 isolated polynucleotide comprising partial or complete PIV background genome or
6 antigenome of a human PIV (HPIV) or bovine PIV (BPIV) combined with one or more
7 heterologous gene(s) or genome segment(s) of a N, P, L, or M gene of a different PIV to
8 form a human-bovine chimeric PIV genome or antigenome.

1 83. The method of claim 82, wherein the chimeric PIV genome or
2 antigenome and the N, P, L and/or M proteins are expressed by two or more different
3 expression vectors.

1 84. An expression vector comprising an operably linked transcriptional
2 promoter, a polynucleotide sequence which includes a partial or complete PIV
3 background genome or antigenome of a human PIV (HPIV) or bovine PIV (BPIV)
4 combined with one or more heterologous gene(s) or genome segment(s) of a N, P, L, or
5 M gene of a different PIV to form a human-bovine chimeric PIV genome or antigenome,
6 and a transcriptional terminator.